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EDITORIAL

3D culture reveals a signaling network

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See related research by Leslie *et al.*, http://breast-cancer-research.com/content/12/5/R80

Abstract

The behavior of a cell is significantly influenced by its context. Epithelial cells derived from glandular organs such as the breast recreate their glandular organization when grown under 3D culture conditions. While traditional monolayer cultures are powerful tools to understand how cells proliferate, grow and respond to stress, they do not recreate the 3D property observed *in vivo*. Multiple studies demonstrate that 3D organization can reveal novel and unexpected insights into the mechanisms by which normal and tumor-derived epithelial cells function. In the present article we comment on a study that reports identification of a RasV12-induced IL-6 signaling network in mammary epithelial cells in 3D cultures.

Some of us may remember conferences with sessions entitled 'Why 3D?' Such sessions are a thing of the past now because there is an accumulating body of evidence – joined by the recent article from Leslie and colleagues [1] – demonstrating the importance and utility of 3D culture systems to discover and model biological process with *in vivo* relevance. For example, when normal and malignant human breast cells are placed in 3D cultures of laminin-rich gels, the former cells form growth-arrested, lumen-containing acini and the latter cells form disorganized structures [2]. Inhibitors of epidermal growth factor receptor and β_1-integrin can 'revert' the malignant phenotype, and each inhibitor downmodulates its own target as well as the other targets only in 3D but not in cells grown as monolayer cultures – suggesting that signaling pathways reciprocally regulate each other to maintain the transformed state [3]. ErbB2/HER2-induced transformation of 3D structures, but not cell proliferation, requires disruption of the Par6 cell polarity pathway [4]. This requirement for deregulation of cell polarity pathways is also observed during ErbB2-induced mammary tumorigenesis in mouse models of human breast cancer [5]. Cells in a 3D matrix can thus provide novel and unexpected insights into cancer biology.

Leslie and colleagues demonstrate an unexpected role for the 3D context in regulating the ability of H-RasV12 to induce IL-6 and activate STAT3 [1]. Consistent with previous reports on the role played by STAT3 [6,7], the authors first demonstrate that downregulation of STAT3 inhibits the ability of H-RasV12 to transform MCF-10A cells. While there was no increase in tyrosine phosphorylation of STAT3 when H-RasV12-transformed MCF-10A cells were grown as monolayer cultures, the authors surprisingly observed a significant increase in phospho-STAT3 and IL-6, a potent activator of STAT3 phosphorylation in tumors derived from H-RasV12-transformed MCF-10A cells. In addition, spontaneous mouse mammary tumors induced by expression of K-Ras under the control of the mouse mammary tumor virus promoter also show an increase in phospho-Stat3 and IL-6.

These observations suggest that STAT3 phosphorylation may be specific to the context in which cells are grown. The authors confirm this possibility by demonstrating that Ras-transformed MCF-10A cells induce expression of IL-6 and activate STAT3 phosphorylation when cultured on a bed of Matrigel or Laminin matrix but not when grown as monolayer cultures [1]. To rule out the possibility that cells in monolayer cultures lost their ability to respond to IL-6, the authors treated the Ras-transformed cells with exogenous IL-6 and demonstrate that the cells possess the ability to induce STAT3 phosphorylation in response to IL-6. They go on to demonstrate that the culture context is critical not only for the activation but also for maintenance of the Ras–IL-6–STAT3 signaling network. Culturing tumor cells from xenograft or Ras-driven primary mouse mammary tumors for a few passages as monolayers results in the cells losing their ability to both express IL-6 and induce phosphorylation of STAT3.

These results, however, differ from a previous report where H-RasV12-transformed SV-40 T/t-Ags and hTERT immortalized human mammary epithelial cells express

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IL-6 even in monolayer cultures [8]. It is possible that an explanation can be offered by considering the differentiation status of MCF-10A and hTERT human mammary epithelial cells. Unlike hTERT immortalized human mammary epithelial cells, which consist of a mixture of cells with epithelial and mesenchymal morphology, the MCF-10A cells have a strict epithelial, cobblestone morphology. Epithelial cells and mesenchymal cells may respond differently to H-RasV12-induced activation of the IL-6–STAT3 pathway. Mesenchymal cells may activate autocrine production of IL-6 in response to expression of oncogenic Ras, whereas epithelial cells do not. Interestingly, the authors show that activation of STAT3 leads to repression of E-cadherin expression and, conversely, inhibition of IL-6 or JAK restored expression of E-cadherin. It is possible that activation of the IL-6–STAT3 signaling pathway induces a loss of epithelial characteristics and pushes cells towards a mesenchymal state.

Consistent with this possibility, previous studies have shown that stimulation of breast cancer cell lines with exogenous IL-6 induces STAT3 activation and down-regulation of E-cadherin to induce epithelial to mesenchymal transition [9]. This process can set up a feed-forward loop where the presence of IL-6 in tumors, produced either by the cancer cells themselves or by immune cells in the tumor microenvironment, can induce tumor epithelial cells to transition to a differentiation state that confers properties such as migration, invasion and resistance to therapy. Not entirely clear, however, is whether the presence of the mesenchymal state is sufficient to couple Ras signaling to activation of the IL-6/STAT3 pathway because K-RasV12-induced transformation of NIH3T3 cells does not induce phosphorylation of STAT3 [10]. Leslie and colleagues show that culturing epithelial cells in a 3D matrix or *in vivo* activates the right nodes in the intracellular signaling network that can now induce the Ras–IL-6–STAT3 network. How is this achieved? A lot remains to be understood.

Given that an increase in circulating IL-6 levels is strongly associated with poor clinical prognosis in patients with breast cancer [11], and that autocrine production of IL-6 promotes multidrug resistance in breast cancer cells [11], the mechanisms by which expression of IL-6 is regulated in breast cancer is of significant clinical importance. The study by Leslie and colleagues further highlights the importance of culture context when investigating signaling networks in breast epithelial cells and emphasizes the importance of using *in vivo* and 3D culture models to verify and validate the observations made in monolayer cultures.

Abbreviations

3D, three-dimensional; hTERT, human telomerase reverse transcriptase; IL, interleukin; JAK, Janus kinase; MCF-10A, Michigan cancer foundation-10A; STAT, signal transducer and activator of transcription; SV-40 T/t-Ags, simian virus large T small t antigens.

Competing interests

The author declares that he has no competing interests.

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